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Abstract: Pollen fate can strongly affect the genetic structure of populations with restricted gene flow and significant inbreeding risk. We established an experimental population of inbred and outbred Silene latifolia plants to evaluate the effects of (i) inbreeding depression, (ii) phenotypic variation and (iii) relatedness between mates on male fitness under natural pollination. Paternity analysis revealed that outbred males sired sig- nificantly more offspring than inbred males. Independently of the effects of inbreeding, male fitness depended on several male traits, including a sexually dimorphic (flower number) and a gametophytic trait (in vitro pollen germination rate). In addition, full-sib matings were less frequent than randomly expected. Thus, inbreeding, phenotype and genetic dissimilarity simultaneously affect male fitness in this animal-pollinated plant. While inbreeding depression might threaten population persistence, the deficiency of effective matings between sibs and the higher fitness of outbred males will reduce its occurrence and counter genetic erosion.

DOI: https://doi.org/10.1098/rspb.2011.0652

Originally published at:
Austerlitz, F; Gleiser, G; Teixeira, S; Bernasconi, G (2012). The effects of inbreeding, genetic dissimilarity and phenotype on male reproductive success in a dioecious plant. Proceedings of the Royal Society B: Biological Sciences, 279(1726):91-100.
DOI: https://doi.org/10.1098/rspb.2011.0652

REVISED VERSION

The effects of inbreeding, genetic dissimilarity and phenotype on male reproductive success in a dioecious plant

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ABSTRACT (150 words)

Pollen fate can strongly affect the genetic structure of populations with restricted gene flow and significant inbreeding risk. We evaluated the effects of (i) inbreeding depression, (ii) phenotypic variation and (iii) relatedness between mates, on male fitness in an experimental population of inbred and outbred *Silene latifolia* plants. Paternity analysis revealed that outbred males sired significantly more offspring than inbred males under natural pollination. Independently of the effects of inbreeding, male fitness depended on several male traits, including a sexually dimorphic (flower number) and a gametophytic trait (*in vitro* pollen germination rate). In addition, full-sib matings were less frequent than randomly expected. Thus, male fitness in this animal-pollinated plant is affected simultaneously by inbreeding, phenotype and genetic dissimilarity to mates. While inbreeding depression might threaten population persistence, the deficiency of effective matings between sibs and the higher fitness of outbred males will reduce its occurrence and counter genetic erosion.

1. INTRODUCTION

During pollination, stigmas may receive pollen from multiple individuals (Marshall & Ellstrand 1985; Bernasconi 2003; Teixeira & Bernasconi 2007) such that pollen tubes compete to fertilize ovules. This promotes the evolution of mechanisms for pollen selection in females that allow the sorting of compatible pollen and increase offspring number or quality, and for selection of male traits that increase attractiveness towards pollinators and pollen-competitive abilities (Mulcahy 1979; Marshall & Folsom 1991; Bernasconi et al. 2004). As plants are sessile, the risk of inbreeding is high, especially when seeds disperse locally and pollination depends on insects, which tend to visit nearest neighbouring plants. The negative effects of inbreeding may be avoided through post-pollination mechanisms of females, such as genetic self-incompatibility, selection of pollen tubes before fertilization or selective abortion of seeds (Hauser & Siegismund 2000; Nasrallah 2002; Skogsmyr & Lankinen 2002; Hiscock & Tabah 2003).

Similarly, when sires contribute to the phenotypes of their offspring for fitness-related traits (e.g. germination time, number and size of flowers, Mazer & Gorchov 1996; Teixeira et al. 2009), pollen recipients may be selected to favour fertilization by pollen donors that provide genetic benefits. Both benefits of good genes and of compatible genes are fundamental to the evolution of pollen receipt and pollen/embryo selection mechanisms, as pollen recipients should favour fertilization by both sires of high genetic quality and genetically dissimilar sires, although the benefits of dissimilarity may follow an optimum curve, given that very dissimilar mates may lead to outbreeding depression (Waser & Price 1989; Waser 1993). Male reproductive success also depends on a

41 combination of different factors, including pollen donor-recipient relatedness (either
42 directly or through phenotypic resemblance, e.g. positive assortative mating for
43 phenology, Gerard et al. 2006), and heritable traits affecting sporophytic vigour, pollen
44 production and attractiveness to pollinators (Oddou-Muratorio et al. 2005). In addition,
45 spatial effects, such as the number of recipient plants in close proximity and the dispersal
46 pattern of pollen, may also affect the fecundity of competing males (Meagher &
47 Vassiliadis 2003; Robledo-Arnuncio & Austerlitz 2006).
48 A sessile life style and the subsequent risks of inbreeding also raise the question
49 whether inbreeding depression may directly influence pollination and siring success.
50 Inbreeding depression that acts late in the life cycle (i.e., at reproduction) can be
51 considerable, because of slightly deleterious alleles that are not easily purged
52 (Charlesworth & Willis 2009). Inbred individuals may for instance flower at an older age,
53 and produce fewer flowers and seeds (Glaettli & Goudet 2006). Moreover, some studies
54 found evidence for inbreeding depression (or hybrid vigor) on pollen traits such as pollen
55 production, pollen viability, number or growth of pollen tubes, and siring success in
56 controlled crosses (Johannsson et al. 1998; Melser et al. 1999; Stephenson et al. 2001;
57 Busch 2005). Since pollen is haploid, dominance relationships as a genetic mechanism
58 underlying inbreeding depression should not affect directly the growth of the pollen tube.
59 Nevertheless, inbreeding depression on pollen expressed traits can result from genetic
60 stress acting in the diploid pollen parent during pollen formation (Stephenson et al. 2001).
61 However, to our knowledge, the impact of the individual level of inbreeding on siring
62 success has never been investigated under conditions of natural pollination.

In this study, we address the determinants of male reproductive success under natural pollination in the dioecious white campion, *Silene latifolia*, using five previously unpublished microsatellite markers to assess paternity. We jointly infer the effects of experimentally controlled levels of inbreeding, male-female relatedness, and of male sporophytic and gametophytic traits on realized male fitness by applying a modified version of the spatially explicit mixed mating model for the analysis of paternity (SEMM, Oddou-Muratorio et al. 2005). This method jointly estimates the pollen dispersal curve, the rate of external pollen flow and the impact on male fitness of phenotypic traits. Combining all of these processes together allows us to disentangle the independent effect of each of these different factors (see Methods for a description of this analytical approach). We specifically ask whether siring success is affected by (i) levels of inbreeding in males, (ii) male-female relatedness, and (iii) male phenotypic traits, including sexually dimorphic and gametophytic traits that may be under sexual selection. Our experimental design consisted of an artificial population composed of males arising from inbred and outbred experimental crosses and exposed to natural pollination.

The white campion is ideally suited to address these questions. First, in *S. latifolia*, the risk for biparental inbreeding is high because of gravity-dispersed fruits and other factors that restrict gene flow (Richards et al. 1999; Richards 2000; Barluenga et al. 2010), while the species frequently occurs in metapopulations with small and isolated sub-populations (Richards 2000). Novel recruits or founder populations appear as a consequence of occasional long-distance dispersal of fruits; these patches often consist of sibships from few or single fruits. This creates a unit of plants sufficiently large to be visible to pollinators (Richards et al. 2003), but increases the risk of inbreeding (Richards

2000; Teixeira et al. 2009). However, it is unknown whether inbred *S. latifolia* males suffer reduced fecundity under open pollination. Second, male-female relatedness affect the proportion of seeds sired by competing pollen donors in hand pollinations (Teixeira et al. 2009) and pollen flow was greater into experimental patches of full-sibs compared to patches of unrelated individuals (Richards 2000). This suggests that post-pollination pollen competition or embryo selection may reduce inbreeding by favoring unrelated males, and therefore reduce the risk of local deme extinction("genetic rescue", Richards 2000; Ingvarsson 2001). However, an experimental design that combines open pollination with variation in male-female relatedness that is independent of inbreeding or allelic variation is required to demonstrate that genetic rescue occurs under natural pollination

Moreover, *S. latifolia* is a suitable species to address phenotypic selection on sexually dimorphic traits, such as flower number (Meagher 1994), which may be under sexually antagonistic selection. Males are likely to produce high flower numbers under sexual selection, as a way to attract more pollinators (Shykoff & Bucheli 1995), while it has been suggested that fecundity selection may favour females with larger and hence fewer flowers because of both a negative genetic correlation between flower size and number and a positive genetic correlation between flower size and ovule number (Meagher 1994; Delph et al. 2004; Steven et al. 2007). Besides, variation for pollen germination is heritable in this species (Jolivet & Bernasconi 2007), suggesting that selection can also occur at the gametophytic phase; therefore, both sporophytic and gametophytic traits should be considered in studies on fitness.

2. MATERIAL AND METHODS

(a) *Study species*

The white campion, *Silene latifolia* (Poiret) (Caryophyllaceae), is a short-lived perennial, entomophilous species, native to Eurasia (Wolfe 2002). The species is dioecious with chromosomal sex determination (Westergaard 1958). Pollinators are mainly moths (Young 2002), including *Hadena bicruris* (Brantjes 1976; Labouche & Bernasconi 2010).

(b) *Creating an artificial population composed of inbred and outbred plants having a similar genetic background*

In 2003, we collected fruits from a population in Village-Neuf (France, 47°36'25"N; 7°33'31E; 245 m a.s.l.). Seeds were germinated and grown in a greenhouse (see details in Teixeira et al. 2009). After all plants had started flowering, we chose 20 females and 36 male plants for crosses. Each female plant was pollinated with pollen from a brother (from the same field-collected fruit as the female plant, i.e. a full- or half-brother) or from a male from a different field-collected fruit from the same population. In the field, each fruit was sampled from a different female and female plants were at least 2m apart from each other. For simplicity, we refer to the latter treatment as cross with an "unrelated male", although it cannot be ruled out that the female and the male, although stemming from different maternal plants, may be related in some cases (e.g. as paternal half-sibs). A previous estimate of relatedness based on three microsatellite loci showed

132 that, as expected, within females the relatedness with brothers was significantly larger
133 than the relatedness with the "unrelated" males (Teixeira et al. 2009). We sowed a
134 random subset of 20 seeds/fruit in Jiffy peat pellets, and recorded the time from sowing to
135 germination and the day when the first flower opened.

136 On day 60 after germination, we measured stem length and placed a subsample of
137 females and males (the latter arising from both outbred and inbred crossings) in a
138 common garden, so as to expose them to natural pollinators during June and July. Sex
139 ratio was of 2:1 (females: males) for a final sample of 342 plants exposed to pollinators
140 (see Supplementary information, Table S1). The spatial arrangement was randomized for
141 gender, inbreeding level and maternal seed family, as shown in Table S1. The
142 experimental population was not isolated from natural populations of *S. latifolia*. We
143 placed the pots at 75 cm inter-plant distance on a mown, flat area. Plants were watered
144 daily. At weekly intervals, for six weeks (i.e. until day 100 from sowing), we counted
145 flowers on all plants to estimate total flower production (estimated as the sum of the
146 number of flowers that were recorded in the censuses). In addition, we assessed *in vitro*
147 pollen germination of the experimental males (see (Teixeira & Bernasconi 2008) for
148 protocol). On day 100, we collected one ripe fruit on 29 female plants (selecting only
149 from among outbred females, see Table S1) and sowed a subsample of the seeds to
150 determine paternity of the seedlings (see below).

151 The field collection of fruits and the crosses with plants derived from them were
152 part of a previous study (Teixeira et al. 2009). The present study differs from Teixeira et
153 al. (2009) in that it includes the genotyping of one additional generation to estimate
154 inbreeding depression for paternal fitness under open pollination, while the previous

155 study estimated inbreeding depression for other vegetative and reproductive traits. Also,
156 both the previous and current study address the effects of male-female relatedness on the
157 proportion of seeds sired, with the difference that this was examined for hand pollinations
158 (controlled crosses) in Teixeira et al. (2009) and for open pollination, i.e. in interaction
159 with naturally occurring pollinating insects, in the present study.
160
161 **(c)** ***Microsatellite genotyping***
162 To infer paternity, we genotyped 29 females, 101 males and 752 seedlings with
163 five nuclear microsatellite markers. We genotyped on average 26 (sd. 10) offspring per
164 female. DNA was extracted from leaf tissue using the Qiagen Biosprint DNA kit. The
165 microsatellite loci were isolated by Ecogenics GmbH (Zurich, Switzerland; see
166 Supplementary information, Table S2). DNA was amplified by PCR (Table S2); the
167 products were separated on an ABI PRISM 3100 genetic analyser (Applied Biosystems),
168 and sizes were assigned with the GENESCAN and GENOTYPER (Applied Biosystems)
169 softwares, using Genescan-350 as the internal size standard.
170 Genetic diversity indices and deviations from Hardy-Weinberg equilibrium were
171 calculated with Genepop 4 (Rousset 2008). The estimations of the frequency of null
172 alleles and exclusion probabilities were estimated with Cervus 3.0.3 (Marshall et al.
173 1998; Kalinowski et al. 2007). The five loci showed a total of 57 alleles in the 133
174 genotyped parents (Table S1), with a mean of 11.4 alleles per locus. The resolution of
175 these markers in parentage analyses was high, as all the individuals could be
176 characterized by a unique multilocus genotype and the cumulative exclusion probability
177 of the second putative father (e.g. the probability of not excluding an unrelated putative

178 father when the maternal genotype is known, Jamieson & Taylor 1997) was 99.6%. All
179 loci showed an excess of homozygotes (Table S1). Departure from the expected
180 heterozygosity was greatest for the loci *Sillat08* and *Sillat28*, partly due to the possible
181 presence of null alleles, as suggested by the comparison of the genotypes of mothers and
182 offspring.
183
184 **(d)** ***Joint estimation of dispersal and male fecundity parameters***
185 The variation in siring success among individuals may result from differences
186 expressed at different times along the life cycle, including pollen grains traits (e.g.
187 quantity, quality and performance), opportunities for mating (e.g. the competition with
188 other sires and the availability of ovules to be sired at the time of flowering), the
189 attractiveness towards pollinators, and also the outcome of the interactions with the
190 females. Paternity analyses constitute the most powerful approach to assess siring success
191 and mating patterns in nature. However, an important limitation of these methods is the
192 potential bias that can arise through pollen immigration from pollen donors outside the
193 sampled area (Devlin & Ellstrand 1990; Oddou-Muratorio et al. 2003) or because some
194 males may sire many seeds not because of their phenotypic advantage, but because of
195 their proximity with females. Recently developed methods eliminate these potential
196 sources of bias by simultaneously assessing differential fecundity of males (regression
197 describing the relationship between male traits and the associated fertilities inferred after
198 paternity assignment), the pollen dispersal curve (allowing thus to correct for the spatial
199 positions of the plants) and the contribution of external gene flow on paternity (Smouse et

al. 1999; Burczyk et al. 2002; Oddou-Muratorio et al. 2005; Burczyk et al. 2006; Gerard et al. 2006).

We analysed siring success by applying the spatially explicit mixed mating model (Oddou-Muratorio et al. 2005; Gerard et al. 2006). This method, which stems from the neighbourhood model (Adams & Birkes 1991; Burczyk et al. 2002), allows the joint estimation of the pollen dispersal curve, the external pollen flow rate (m) and the impact of several phenotypic or ecological traits on the male fecundity using a maximum likelihood procedure. It uses the genotypes of potential fathers, mothers and offspring (seeds), their spatial position and their phenotypic measures. We adapted the method for dioecious species by removing the possibility of selfing and by considering only the males as potential fathers. As the exponential power kernel (Clark 1998) performed poorly here (results not shown), we assumed a geometric dispersal kernel, where the probability for a pollen grain to disperse at positions (x, y) assuming that the father is at position (0,0) is given by

$$p_g(a,b;x,y) = \frac{(b-2)(b-1)}{2\pi a}\left(1+\frac{r}{a}\right)^{-b} \quad (1)$$

with $r = \sqrt{x^2+y^2}$. a represents the scale parameter (the extent to which pollen will disperse) and b the shape parameter of the dispersal curve, which describes the tail of the distribution: the lower b is, the higher the proportion of long-distance dispersal (see Austerlitz et al. 2004 for details).

We estimated jointly these dispersal parameters, along with the impact on the fecundity of males of various factors. We treated the level of inbreeding (males arising from inbred crosses vs. arising from outbred crosses) as a qualitative factor. In addition, five phenotypic traits of the males were treated as quantitative factors: germination time (mean 7.31 days, sd 2.56 days), flowering age (mean 46.31 days, sd 4.75 days), pollen *in vitro* germination rate (mean 26.04%, sd 7.31%), length of the stems at day 60 (mean 57.27cm, sd 11.90cm), total number of flowers (mean 61.86, sd 26.81). Finally, we considered relatedness between males and females as a qualitative factor with four levels (unrelated, half-cousins, half-sibs or full-sibs vs mating partners). Relatedness was inferred from the crossing design using the three-generation pedigree information. This factor differs from the others, because its value for a given male depends on the female with which it mates; we thus modified the algorithm developed by Oddou-Muratorio et al. (2005) accordingly.

For qualitative factors, we set the fecundity of one of the classes to 1 and estimated the relative fecundity of the other classes. For quantitative factors, we estimated their impact on fecundity by assuming a linear selection gradient (Lande & Arnold 1983; Smouse et al. 1999; Wright & Meagher 2004), where the fecundity $f_i(z_{ki})$ for a male m with phenotypic value z_{mi} at trait i is given by the relation:

$$\ln(f_i(z_{mi})) = \beta_i z_{mi} \qquad (2)$$

where β_i denotes the regression coefficient; a positive value of β_i indicates that the trait under consideration is under directional selection for higher values. As in previous works

(Burczyk et al. 2002; Wright & Meagher 2004; Oddou-Muratorio et al. 2005), we assumed that the fecundities for qualitative and quantitative traits were multiplicative; i.e. that the fecundity of a male individual m with phenotypic value z_{mi} at trait i and z_{mj} at trait j was $f_i(z_{mi})f_j(z_{mj})$. The significance of each estimated parameter was tested by performing a likelihood ratio test, comparing the likelihood for the model excluding each parameter one at a time and the likelihood of the full model (Oddou-Muratorio et al. 2005). Confidence intervals for the estimated parameters of fecundity were calculated by decreasing the maximum log-likelihood value by 2 units in order to establish the boundaries at 95% (Kaplan et al. 1995).

3. RESULTS

(a) *Inbreeding and male-female genetic similarity as determinants of siring success*

The experimentally controlled level of inbreeding of the males had a strong and significant effect on their siring success: when exposed to naturally occurring pollinators, outbred males sired significantly more offspring than inbred males (Table 1, Fig. S1). The outbred males had on average a relative fecundity of 1.61 (95%-CI: 1.31, 2.00), compared to inbred males (fecundity set to 1). In addition to levels of inbreeding, the genetic relatedness between mating partners also significantly predicted siring success (Table 1, Fig. S2). Male plants were less successful at siring the offspring of their full sisters than males not or less closely related to females. Half-cousins, on the other hand, appeared to have slightly more siring success than unrelated males; however, this

tendency only approached significance, as the 95% confidence interval of their siring success included the value 1.0 (equal relative fertility compared to unrelated males).

(b) ***Male phenotypic traits as determinants of siring success***

Males with higher siring success were taller, germinated earlier, flowered later, produced more flowers (Table 1, Fig. S3), and produced pollen with higher rates of *in vitro* pollen germination. Because of the statistical model used, the estimated effects of phenotype on paternal fitness were independent of the effects of inbreeding on the phenotypic traits (for an estimate of inbreeding depression of inbred and outbred fathers, see Teixeira et al. 2009), i.e. they indicate effects of phenotypic differences among males that act in addition to those mediated by inbreeding depression on the traits under consideration. Most notably, these results suggest that positive selection may be acting on increased flower production. Since we estimated flower production over six weeks of pollinator exposure, we assume here that it approximates reliably the number of open flowers in males at the time of pollination. Interestingly, flower number is a strongly sexually dimorphic trait in *S. latifolia*

Among the five male traits that we considered, only germination time and age at first flowering were significantly correlated (Spearman rank test, r_s=0.40, $p < 0.001$; Table S3) and none of these five traits showed significant differences between inbred and outbred individuals (Wilcoxon test, all p-values>0.16; Table S4). Note that as two of these traits ("Germination time (days)" and "Age at first flowering (days)") diverged significantly from normality (Shapiro-Wilk test $p = 0.00571$ and $p = 1.14\times10^{-13}$ respectively), we could only perform non-parametric tests. Nevertheless, even if two

289 variables are correlated, their effects on siring success are independent of each other, as
290 each predictor was removed stepwise from the full model and all the LR tests performed
291 were significant (see Methods). Thus, the significance of these phenotypic effects (as
292 well as all other factors considered simultaneously in the model) cannot be attributed to
293 correlations among them, and each of the phenotypic traits with significant effect
294 contributed independently to the variation in siring success. The summed effects of all
295 measured traits yielded a strong heterogeneity in the estimated individual male
296 fecundities (Fig. 1).
297
298 **(c)** ***Assortative mating for flower number***
299 We found evidence of assortative mating only for flower number (Supplementary
300 Information, Table S5) such that males with more flowers tended to mate with females
301 with more flowers. No significant patterns of assortative mating were found for
302 germination time, age at flowering, or stem length (Table S5).
303
304 **(d)** ***Pollen dispersal curve***
305 The SEMM model also allowed us to estimate the parameters of the dispersal
306 kernel (Table 1 and Figure S4). The shape parameter (b) of the geometric dispersal kernel
307 was very low ($b = 0.715$), indicating a fat-tailed dispersal curve, consistent with the
308 occurrence of many short-distance dispersal events and a few long-distance dispersal
309 events. The rate of external pollen flow (m) was estimated at around 15%.
310

4. DISCUSSION

Pollen fate within plant populations can depend on several factors, including pollinator behaviour, spatial structure, plant phenotype, etc. For plants that often occur in small patches, an important aspect is whether the level of inbreeding of individual plants and their degree of relatedness, will also impact this pollen fate, since this will have a major effect on the number, quality and genetic variability of the offspring produced. Here we examined whether inbreeding, relatedness among mates and plant phenotype affected male fitness in an experimental population in which spatial structure was randomized and accounted for statistically.

(a) *Inbreeding depression for siring success in* Silene latifolia

Inbreeding depression has been demonstrated to occur for several traits. The novelty of this study is that it provides direct experimental evidences that inbreeding strongly decreased male fitness (siring success) under ecologically relevant conditions of natural pollination, as it was demonstrated here in *Silene latifolia*. The estimated fecundity of outbred males was more than 1.5 times higher than that of inbred males. This has important implications in this species with metapopulation dynamics. In a patch with inbred and outbred individuals, outbred individuals will often result from recent pollen flow into the patch, which often initially consist of related plants due to gravity dispersal of fruits (Barluenga et al. 2010). In this context, that outbred individuals are expected to enjoy a greater reproductive success, and may contribute to genetic rescue (see below).

This observed higher siring success of outbred males cannot be an artifact due to differences in genetic background between inbred and outbred males, since both were derived from controlled crosses using the same sets of families. Nor can maternal environmental effects, which were eliminated by rearing plants for one generation under greenhouse conditions, be responsible. Maternal genotypic effects on siring success were also controlled by nesting the two pollination treatments (inbred and outbred) within each mother. In addition, we also controlled for spatial effects by using a spatially explicit mixed-mating model that allowed us to disentangle the relative impact of the dispersal patterns and of the phenotypic traits on male fecundity (Oddou-Muratorio et al. 2005).

Our results are consistent with previous studies that reported inbreeding depression for traits that may contribute to siring success, such as *in vitro* pollen germination (Johannsson et al. 1998; Stephenson et al. 2001) and for pollen performance of inbred and outbred pollen donors in controlled pollinations (Melser et al. 1999), including *S. latifolia* (Teixeira et al. 2009). However, these experimental approaches may not always reflect pollination patterns under natural conditions, especially in entomophilous species. By exposing experimental plants to natural pollination, we demonstrated that inbred males obtained substantially lower reproductive success than outbred males, through the effect of inbreeding on traits that may be, for instance, involved in pollinator attraction, flower and pollen production, or pollen performance and competitive ability.

(b) ***Effect of genetic dissimilarity between males and females on siring success***

We found that the degree of relatedness between males and females significantly affected mating success. Matings between full sibs were significantly less frequent than matings between unrelated individuals. This was suggested indirectly in a previous study (Richards 2000), where greater rates of external pollen flow were found in experimental *S. latifolia* patches consisting of full sibs than in patches consisting of unrelated individuals. Moreover, in pollen competition crosses between an unrelated and a related male, the siring success of unrelated males was greater when the competing male was more closely related to the maternal plant (Teixeira et al. 2009). Previous studies also show significant male genotype x female genotype interactions on siring success (Teixeira et al. 2008). Importantly, our study provides direct evidence that this also occurs under natural pollination. Siring success was estimated here from seedlings, so we cannot discern whether full-sib mating events are underrepresented due to female choice, early-acting inbreeding depression yielding within-fruit selective abortion of seeds, or the lower competitive ability of inbred seeds within fruits. While the exact mechanism remains to be evaluated, the net effect is to reduce the number of inbred offspring produced.

Unlike full sibs, half-cousins had higher siring success than unrelated individuals (Fig. S2). We are cautious about this result given that our results on this aspect only approached significance. However, both genetic models (Schierup & Christiansen 1996), and empirical data (see for example Grindeland 2008) suggest that in many species there is selection for an optimal outcrossing distance. While our results show that seedlings arising from crosses between full sibs in *S. latifolia* are underrepresented, the potential

existence of outbreeding depression or of selection for intermediate levels of inbreeding within populations deserves further investigation.

(c) ***Implications for genetic rescue***

Interestingly, "genetic rescue" has been invoked for *S. latifolia* as a mechanism for population persistence (Richards 2000; Ingvarsson 2001). Since immigrant genes will lead to outbred progeny within demes, the higher fecundity of outbred males and deficiency of successful matings between closely related partners that we observed would suggest that such a "genetic rescue" through greater reproductive success of immigrant alleles would persist or even be reinforced in the generations that follow the immigration event.

(d) ***Implications for the evolution of unisexuality in*** **Silene**

The presence of inbreeding depression for male fitness in *S. latifolia* provides further insights in the evolution of unisexuality that occurred within the genus (e.g. dioecy was found to be a derived sexual system, Desfeux et al. 1996). It has been proposed that inbreeding depression can play an important role in the evolution and maintenance of unisexuality in angiosperms, both in theoretical (Charlesworth & Charlesworth 1978) and in empirical (Dorken et al. 2002; Weller & Sakai 2005) studies. In the genus *Silene* in particular, some species are gynodioecious, and the females in these populations could have appeared as a consequence of selection against inbreeding. In fact, high levels of inbreeding depression have been found in *S. vulgaris*, a gynodioecious species with nucleocytoplasmic sex determination (Glaettli & Goudet

2006). The transition from gynodioecy to dioecy in this group is still not understood, but the hypothesis of selection for inbreeding avoidance leading to dioecy cannot be ruled out provided that the selfing rates in hermaphrodites are high (see Dorken et al. 2002 for an example of dioecy evolving as a consequence of inbreeding avoidance). Nevertheless, inbreeding depression by itself is not enough to account for the complete evolution of dioecy (Charlesworth & Charlesworth 1987; Freeman et al. 1997), and other mechanisms should also be involved in this evolutionary transition. Comparative studies among populations differing in sexual system would be very valuable in order to understand this issue.

(e) ***Phenotypic selection on male fecundity***

Similar to previous studies (Wright & Meagher 2004), we found substantial variation in male reproductive success under natural pollination, with increased success correlated with several traits. In particular, we showed that one major trait that increases male reproductive success is the number of flowers, and this may be a consequence of an increased pollen production (Delph et al. 2004) or pollinator attraction (or both). Indeed, it has been shown that males with larger floral displays receive more pollinator visits (Shykoff & Bucheli 1995). In our study, we estimated flower number as the total flower production over six weeks of exposure of the plants to pollinators. Thus, we assumed here that our estimate of flower production was correlated with display size at the time of pollination. We believe that this assumption is reasonable since the six weeks of exposure are a relatively short part of the flowering season (moreover, it takes approximately four weeks between pollination and fruit ripening) and all fruits were collected at the same

time, on the last day of exposure. In future studies, it would be interesting to obtain a direct and more exact estimate of the actual flower display size at the time of pollination (which can controlled, for instance, through bagging of floral buds before exposure).

The finding that male fitness increases with the increase in the number of male flowers has important implications for understanding the evolution of sexual dimorphism, a major research topic in *S. latifolia* (Meagher 1992; Delph et al. 2004; Delph et al. 2005). It has been suggested that the evolution of sexual dimorphism may be driven by selection for increased flower number in males (Delph et al. 2004; Steven et al. 2007), the most extremely dimorphic trait (Delph et al. 2002). For selection to occur, genetic variation should translate into differences in fitness. By growing plants under controlled conditions, we controlled for environmental variation, so that differences in floral display among males should reflect genetic variation. Our study, therefore, shows that a genetically determined increase in male flower number increases fitness, providing evidence that this trait is under selection in *S. latifolia*. In addition to this, there was assortative mating for the number of flowers produced, which is also consistent with a role of pollinators in the response to larger floral displays (Table S5).

Increased siring-success was also explained by other male sporophytic traits: larger plant height, later flowering, and earlier germination. Taller plants may increase siring success by attracting more pollinators, as was shown, for instance, in *Sorbus torminalis* (Oddou-Muratorio et al. 2005), while the effect of flowering time may be a consequence of increased phenological overlap with females. The effect of germination time is best explained by correlations with other traits (Katharina Foerster and G. Bernasconi, unpublished data) that influence pollinator behaviour, such as nectar quantity

446 or quality. While the proximal causes of this effect remain to be studied, the corollary of
447 this result is that genetic variation in early-history traits can translate into fitness
448 differences reflected later on as differences in the siring success of the individuals.
449 We also found that males producing pollen with higher *in vitro* germination rates
450 had greater male fecundity. By performing the germination assay *in vitro* we removed the
451 effects of interactions with females on germination success. Interestingly, pollen
452 germination rate is a trait expressed at the gametophytic phase. Most paternity studies
453 performed in open-pollination have focused on sporophytic traits (Burczyk et al. 2002;
454 Oddou-Muratorio et al. 2005; Gerard et al. 2006). Pollen traits such as tube growth rate
455 can display heritable variation, and this is the case for *in vitro* pollen germination rate in
456 *S. latifolia*, (Jolivet & Bernasconi 2007; Teixeira & Bernasconi 2008). The positive
457 relationship between *in vitro* pollen germination and the proportion of seeds sired may
458 be the result of gametophytically expressed genes, or genes expressed in the sporophytic
459 stage that influence pollen efficiency (e.g. resources stored in the pollen grain,
460 Stephenson et al. 2001). In *S. latifolia*, fruits are often sired by many fathers, suggesting
461 that pollen competition is very likely (Teixeira & Bernasconi 2007). Our result that
462 higher rates of pollen germination increase siring success is consistent with the finding
463 that pollination timing strongly affects proportion of seeds sired (Burkhardt et al. 2009).
464 In conclusion, this study provides experimental evidence for inbreeding
465 depression on siring success in a dioecious species with a meta-population structure,
466 limited gene flow and high risk of biparental inbreeding. Further, the offspring of full-sib
467 matings were significantly less represented than offspring of crosses between unrelated
468 mates, consistent with post-pollination pre- or post-zygotic selection against inbred

469 matings. Since outbred males with greater siring success are likely to be the offspring of
470 recently immigrated pollen, these two effects may mitigate genetic erosion within small,
471 isolated sub-populations. In addition, as predicted by theory, our paternity analysis
472 provides direct evidence that flower number (here estimated as flower production over
473 six weeks of experimental exposure to pollinators), the most sexually dimorphic trait in
474 this species, is under positive selection in males. We found selection on several different
475 traits of the male phenotype, including pollen germination rate, a gametophytic trait.
476 Altogether, our study indicates that pollen fate within plant populations depends on
477 genetic makeup. The patterns of realized paternity within demes may reduce inbreeding
478 by favouring outbred individuals, and the mating between unrelated individuals.
479 Simultaneously, independently of the effects of inbreeding on plant phenotype, several
480 male traits can simultaneously be under selection, including sexual selection.
481

482 **ACKNOWLEDGEMENTS**

483 We thank Sara Carlson, Lynda Delph, John Pannell, Jacqui Shykoff, Yvonne Willi,
484 Laurence Harder and two anonymous reviewers for valuable comments on the manuscript.
485 We thank Marta Barluenga for a preliminary analysis of offspring genotypes, and Anne
486 Burkhardt, Rui Candeias and Katharina Foerster for practical help. This study was supported
487 by the Swiss NSF (no. 3100A0-122004/1 to GB; PIOIA-119443 and IZK0Z3-125693 to FA
488 and GB), the Fondation Mercier pour la Science (to GB) and the Forschungskredit of Zurich
489 University (560065 to GB).
490

491 **Competing interests.** The authors have declared that no competing interests exist.

Author contributions.

GG optimized and characterized the microsatellite loci and conducted all genetic analysis and part of the data analysis. FA adapted the statistical models and conducted statistical analyses. ST conducted all crosses, monitored the experimental population, and phenotyped all plants. GB conceived and designed the experiments. GG, FA and GB contributed to writing.

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673

674 **Table 1.** Estimated parameters of pollen dispersal and male fecundity in the experimental *S. latifolia* population exposed to natural
675 pollinators. The algorithm jointly estimates the pollen immigration rate (*m*), the scale (*a*) and shape (*b*) parameters of the pollen
676 dispersal curve, and the impact of male-female relatedness and male traits on fecundity. For qualitative traits, the fecundity of one of
677 the classes is fixed to be 1 (the fixed classes were "inbred" for the level of inbreeding, and "unrelated" for male-female relatedness),
678 thus estimates represent relative fertilities. For quantitative traits, the estimated effects are regression slopes. The significance of each
679 factor was tested by removing the factors each by each from the full model and by comparing the reduced models with the full model
680 by a likelihood ratio (LR) test. The boundaries of the 95% confidence intervals are shown in brackets.
681

Parameter/Effect	Estimate	- Log-likelihood	*p*- value
m (pollen immigration rate)	0.153 (0.124, 0.185)		
a	0.004 (0, 0.701)		
b	0.715 (0.605, 0.823)		
Level of inbreeding (outbred vs inbred male)	1.62 (1.31, 2.00)	5615	$< 10^{-4}$
Male-female relatedness		5612	0.006
half-cousins vs unrelated	1.55 (0.994,2.28)		
half-sibs vs unrelated	1.46 (0.798, 2.40)		
full-sibs vs unrelated	0.256 (0.043, 0.73)		
Male phenotypic traits			
Germination time (days)	-0.079 (-0.125, -0.039)	5611	0.002
Age at first flowering (days)	0.040 (0.020, 0.059)	5611	0.003
Pollen germination in vitro (%)	0.015 (0.002, 0.029)	5608	0.033
Stem length (cm)	0.014 (0.005, 0.022)	5611	0.002
Total number of flowers	0.006 (0.0025, 0.010)	5611	0.002
Full model		5606	

682

683 **FIGURE CAPTIONS**

684

685 **Fig. 1.** Estimated individual male fecundities of the 101 genotyped males, normalized so

686 that the average male fecundity equals 1.0, and ranked by increasing fecundities.

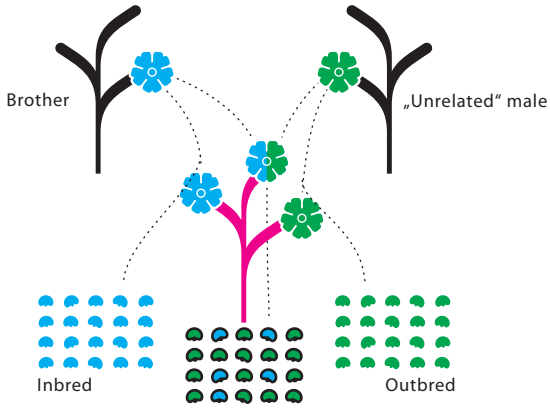
687

688

689

Teixeira et al. 2009 Heredity

Experimental crosses using F1 plants



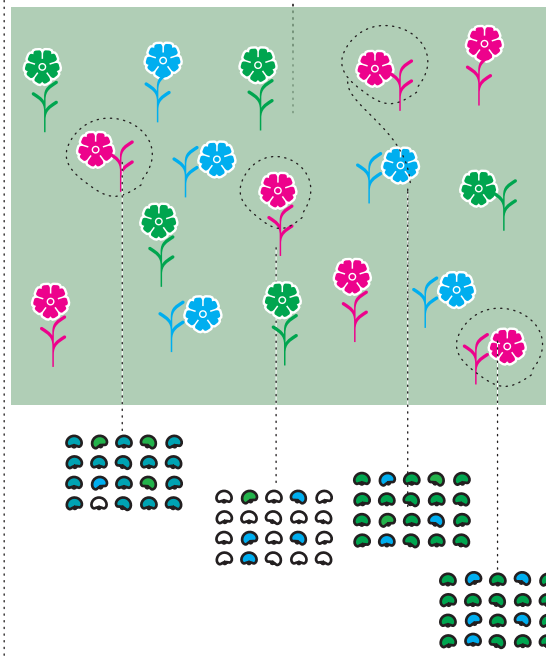
Paternity analysis by genotyping F2 plants

Conditions:
Hand pollination
Pollen from two donors
Constant pollen load size

Examined predictors:
Genetic dissimilarity between mates

Present study

Expose F2 plants to pollinators in a common garden



Paternity analysis of open-pollinated fruits by genotyping F3 plants

Conditions:
Natural pollination
Pollen from N donors
Variable pollen load sizes

Examined predictors:
Inbreeding level of pollen donor
Genetic dissimilarity between mates
Male phenotype (sporophytic and gametophytic components)
Pollen dispersal

120 x 210mm

Table S1

Spatial arrangement of *Silene latifolia* plants in the experimental population, by gender, level of inbreeding, and maternal seed family. *NN*=plants from mixed pollination crosses (see Teixeira et al. 2009) for which genetic paternity assignment failed. The female plants included in the paternity analysis are surrounded by an square and highlighted in bold.

North	A	B	C	D	E	F	G
1	male inbred 3,20	male inbred 11.13	male inbred 14.8	female outbred 1.14	**female outbred 11.13**	female outbred 11.13	female NN 5.19
2	female outbred 10.16	male inbred 10.16	male outbred 5.19	male inbred 8.12	female outbred 14.8	female outbred 1.7	male outbred 7.7
3	female inbred 6.15	male outbred 1.7	male outbred 1.7	female inbred 14.8	**female outbred 4.12**	female inbred 1.14	female inbred 4.6
4	male inbred 4.6	male inbred 3,20	male NN 11.13	female inbred 10.16	male outbred 1.14	female inbred 9.8	female inbred 11.6
5	female outbred 14.8	female outbred 5.19	male inbred 15.14	**female outbred 14.16**	**female outbred 3,20**	female inbred 9.8	male inbred 11.6
6	female inbred 15.9	female outbred 4.12	male inbred 8.11	male outbred 6.15	male outbred 10.16	female outbred 3,20	female inbred 15.14
7	female inbred 9.8	male inbred 15.14	female outbred 1.7	**female outbred 9.8**	female outbred 1.14	**female outbred 8.12**	female inbred 1.14

8	female outbred 7.7	male outbred 6.15	female inbred 3,20	female outbred 10.16	male inbred 15.9	female inbred 4.12	male outbred 11.13
9	male inbred 3,20	female outbred 15.9	male outbred 4.12	female inbred 11.6	**female outbred 1.14**	**female outbred 15.14**	female outbred 8.11
10	female inbred 15.14	female outbred 14.8	male outbred 14.16	female inbred 8.12	female outbred 9.8	female inbred 15.9	female outbred 1.7
11	male inbred 14.8	female inbred 1.14	female inbred 3,20	female inbred 7.7	**female outbred 3,21**	female outbred 8.11	male inbred 11.6
12	male outbred 14.16	female outbred 4.6	female inbred 1.7	female inbred 7.7	male inbred 7.7	female outbred 7.6	female outbred 14.8
13	male outbred 11.6	male inbred 9.8	female inbred 7.7	female inbred 3,20	male outbred 15.14	female inbred 3,20	male inbred 10.16
14	**female outbred 11.6**	female outbred 4.6	male outbred 7.6	female outbred 10.16	female NN 6.15	female inbred 8.11	male inbred 15.9
15	male inbred 1.7	male outbred 4.6	female inbred 3,20	female inbred 4.6	male outbred 15.9	**female outbred 14.16**	female outbred 3,20
16	female inbred	female outbred	male inbred	female outbred	male outbred	male inbred	male inbred

	3,20	14.16	1.14	1.7	4.6	8.11	14.16
17	female inbred 6.15	female inbred 11.6	female outbred 4.6	female outbred 4.12	female inbred 15.14	female outbred 3,20	male outbred 3,20
18	male inbred 7.7	female inbred 8.11	female outbred 6.15	female outbred 7.6	male inbred 9.8	**female outbred 5.19**	female inbred 8.12
19	female outbred 14.16	male outbred 8.12	female inbred 7.7	male outbred 4.12	female inbred 4.6	male inbred 7.6	female inbred 8.11
20	male outbred 5.19	female inbred 5.19	female inbred 14.16	male inbred 4.12	female inbred 7.6	female outbred 7.6	male outbred 8.11
21	female outbred 7.6	female inbred 14.8	female inbred 8.11	female inbred 14.16	female inbred 8.12	female inbred 15.9	male inbred 6.15
22	female inbred 1.7	female inbred 15.9	male inbred 1.14	**female outbred 15.9**	female inbred 8.12	male outbred 7.6	female inbred 7.6
23	female outbred 6.15	female inbred 5.19	female outbred 11.13	**female outbred 7.7**	male inbred 5.19	female inbred 9.8	male outbred 14.8
24	female outbred 11.6	male inbred 3,20	female outbred 3,20	male inbred 8.12	female outbred 3,20	female outbred 8.12	female outbred 5.19
25	female	**female**	female	male	male	male	male

	outbred 10.16	**outbred** **6.15**	outbred 11.13	outbred 9.8	inbred 8.12	inbred 8.11	outbred 7.6
26	male outbred 7.7	female outbred 1.7	female inbred 5.19	female outbred 14.8	**female** **outbred** **8.11**	female outbred 15.14	male inbred 11.13
27	female NN 10.16	female outbred 8.11	female outbred 8.12	male outbred 11.13	male NN 5.19	male inbred 1.14	female inbred 5.19
28	male NN 11.6	female outbred 7.6	female inbred 7.7	male outbred 14.16	female inbred 8.11	female NN 15.14	male outbred 11.6
29	female inbred 10.16	female outbred 11.13	female outbred 6.15	female outbred 14.8	female inbred 3,20	female inbred 1.14	male outbred 8.11
30	female outbred 4.12	female outbred 4.6	male inbred 8.12	female inbred 4.6	male outbred 8.11	female outbred 11.13	male inbred 14.16
31	**female** **outbred** **3,20**	male outbred 7.6	female outbred 14.16	female inbred 4.12	**female** **outbred** **5.19**	female inbred 5.19	**female** **outbred** **7.7**
32	female outbred 9.8	female inbred 8.12	female inbred 15.9		male inbred 3,20	male inbred 15.14	female outbred 14.16
33	female inbred 8.11	female inbred 7.6	male inbred 5.19	female outbred 15.9	female NN 3,20	male outbred 4.12	female outbred 6.15

34	male inbred 1.14	female inbred 7.7	male outbred 14.8	female inbred 6.15	female inbred 1.14	**female outbred 1.7**	female outbred 14.8
35	female inbred 9.8	**female outbred 10.16**	female inbred 9.8	male outbred 15.9	**female outbred 11.6**	female outbred 5.19	female inbred 15.14
36	female outbred 11.6	female outbred 1.14	female inbred 15.9	male inbred 7.6	female inbred 3,20	male inbred 1.7	male inbred 6.15
37	female outbred 14.16	male outbred 8.12	**female outbred 1.7**	female inbred 1.7	female outbred 14.16		female outbred 14.8
38	male inbred 3,20	female inbred 8.12	female inbred 6.15	female inbred 7.6	female outbred 7.7	male outbred 10.16	female inbred 1.7
39	female inbred 7.7	male NN 11.13	male NN 1.7	**female outbred 8.12**	female inbred 9.8	female outbred 3,20	female inbred 7.7
40	male outbred 15.14	male inbred 15.9	male inbred 15.9	female outbred 7.6	female inbred 8.12	female outbred 7.6	male outbred 14.8
41	female inbred 14.16	male inbred 14.8	male outbred 15.14	female inbred 8.12	female inbred 3,20	male NN 6.15	female outbred 4.6
42	female NN 10.16	female inbred 3,20	female inbred 15.14	female inbred 14.16	male inbred 9.8	male inbred 11.6	male outbred 6.15

43	female outbred 6.15	male inbred 10.16	male inbred 10.16	female inbred 1.14	male outbred 5.19	female outbred 1.7	female inbred 11.6
44	male outbred 4.12	female inbred 14.8	female inbred 8.11	female inbred 11.6	female inbred 1.14	female inbred 15.9	female inbred 15.9
45	female NN 3,20	male inbred 7.7	female outbred 4.6	female inbred 14.8	male outbred 4.6	male inbred 3,20	male outbred 9.8
46	male inbred 3,20	**female outbred 6.15**	female inbred 4.6	**female outbred 5.19**	female outbred 4.6	male outbred 14.16	female inbred 3,20
47	female outbred 10.16	male inbred 4.6	female outbred 15.9	female outbred 3,20	female outbred 4.12	**female outbred 9.8**	female outbred 1.14
48	female inbred 11.6	female outbred 11.13	male outbred 1.7	male outbred 4.6	male NN 7.7	female outbred 3,20	female outbred 7.6
49	male outbred 9.8	male outbred 3,20	male inbred 4.12	female NN 9.8	male outbred 1.14	**female outbred 11.13**	**female outbred 8.11**
50	male outbred 3,20						

Table S2. Characterization of five newly-isolated microsatellite loci in *Silene latifolia*. The regions were isolated from $(GT)_{13}$, $(CT)_{13}$, $(GTAT)_7$ and $(GATA)_7$ enriched libraries. Primers were designed for positive clones, and five primer-pairs were chosen according to their levels of polymorphism. Forward primers were labelled with fluorescent dyes (<6-FAM>, <HEX>) for automated electrophoresis. PCRs were conducted in a 10 µl mix containing approximately 5-10 ng of template DNA, 2 µM each of the forward and reverse primers, and 1x Qiagen HotStarTaq *Plus* Master Mix. Amplification consisted of an initial activation step of 15 minutes at 95 ºC, followed by *n* cycles of 30 s of denaturation at 94 ºC, 90 s of annealing at *T*, 60 s of extension at 72 ºC, and a final extension step of 30 min at 60 ºC. The table shows the locus name, primer sequence (F: forward primer, R: reverse primer), repeat motif, annealing temperature (*T*), number of cycles in the PCR (*n*), number of alleles (N_A), allele size range, observed (H_O) and expected (H_E) heterozygosities, F_{IS}, estimated null allele frequencies (F_{null}), exclusion probabilities when one parent (mother) is known (*EP*), and GenBank accession numbers. All genetic parameters were estimated in the parental population used in this study. Data are presented for 133 genotyped individuals (104 males and 29 females).

Locus	Primer sequence (5'-3')	Repeat motif	*T* (ºC)	*n*	N_A	Allele size range	H_O	H_E	F_{IS}	F_{null}	*EP*	GenBank number
Sillat06	F: <6-FAM>CGCTCGAGAATAAGAGAGTCG R: GCACACTCCCCTTTCCTAAC	$(GA)_{24}$	56	27	13	167-225	0.770	0.840	0.083^{ns}	0.046	0.684	GU562854
Sillat07	F: <HEX> ACCGAGTATCGCGGCTGTTA R: GAGTAGGGAGGTGCGTGGTA	$(TC)_{24}$	60	29	14	147-211	0.772	0.883	0.126**	0.067	0.760	GU562855
Sillat08	F: <6-FAM>AAATGTAAACACCCTTATGAAGAAAG R: GATCAACAGAGGGATATATTATGAGG	$(GTAT)_6$	50	27	6	106-134	0.423	0.787	0.464**	0.297	0.577	GU562856
Sillat25	F: <HEX> CGGAATTTATTTCATTTCTACACC R: ATCATGACGCAACTCCGTTC	$(GA)_{25}$	56	25	16	174-242	0.784	0.885	0.115*	0.060	0.765	GU562857
Sillat28	F: <6-FAM> TCGAGTCAGTGTTCCTTCGTC R: AAGCACCACACTTAAAGGAAAAC	$(GT)_{17}$	56	25	8	65-100	0.482	0.671	0.283**	0.167	0.457	GU562858

** $P < 0.01$; * $P < 0.05$; ns, not significant

Table S3
Matrix of Spearman rank correlations (above the diagonal) and their associated *p* values (below the diagonal) for the five traits considered.

	Germination time (days)	Age at first flowering (days)	Pollen germination in vitro (%)	Stem length (cm)	Total number of flowers
Germination time (days)		0.401	-0.185	0.111	0.121
Age at first flowering (days)	3.28×10^{-5}		-0.111	0.183	0.082
Pollen germination in vitro (%)	0.068	0.275		-0.022	0.045
Stem length (cm)	0.271	0.068	0.829		-0.055
Total number of flowers	0.229	0.412	0.659	0.585	

Table S4

p values of the Wilcoxon tests used for testing the difference between inbred and outbred males for the five traits considered.

Trait	*p* value
Age at first flowering (days)	0.82
Stem length (cm)	0.32
Total number of flowers	0.17
Pollen germination in vitro (%)	0.55
Germination time (days)	0.16

Table S5.
Estimated parameters of assortative mating in the experimental *S. latifolia* population exposed to natural pollinators. A similarity index was defined for each quantitative trait measured in both males and females; significant positive estimates indicate assortative mating. The statistical significance of each effect was tested by a likelihood ratio (LR) test that compared the full model with a nested model that lacked the factor being tested. *a*: scale parameter; *b*: shape parameter (see Table 1); *m*: pollen immigration rate. Upper and lower limits of the confidence intervals at 95% are shown between brackets. For details of calculations, see (*).

Parameter/Variable	Estimate	- Log-likelihood	*p*- value
m (pollen immigration rate)	0.153 (0.124, 0.185)		
a	0.168 (0, 0.939)		
b	0.723 (0.611, 0.834)		
Germination time (days)	-0.083 (-0.203, 0.016)	5630	0.126
Age at first flowering (days)	0.086 (-0.017, 0.188)	5630	0.115
Stem length (cm)	-0.027 (-0.133, 0.080)	5629	0.615
Total number of flowers	0.274 (0.156, 0.389)	5640	$< 10^{-5}$
Full model		5629	

(*) We modified the spatially explicit mating model to gauge the level of assortative mating between males and females for the four quantitative factors that were measured on both males and females (germination time, flowering age, length of the stems at day 60 and total number of flowers). For this purpose, we standardised the phenotypic values of given males m for a trait *i*, z_{mi}, as

$$z'_{mi} = \frac{z_{mi} - \bar{z}_{mi}}{sd(z_{mi})}$$

where $\bar{z}_{mi}$ is the average value for the trait across all males and $sd(z_{mi})$ is the standard deviation. Similarly, we standardized the phenotypic traits of each female *f* as

$$z'_{fi} = \frac{z_{fi} - \bar{z}_{fi}}{sd(z_{fi})}.$$

We defined then a standardised similarity index s_{mfk} between a male *m* and a female *f* at trait *i* as

$$s_{mfi} = z'_{mi}\, z'_{fi},$$

that will be positive if the male and the female have both low or high phenotypic values for the trait, and will be negative if one has a high phenotypic value and the other a low one. We assumed as above a log-linear model, in which the fecundity of a male *m* over a female *f* that share a similarity index s_{mfk} for a trait *i* is given by

$$\ln(f_i(s_{mfi})) = \gamma_i z_{mfi},$$

and we estimated the γ_i coefficients using the same likelihood method as described above; a positive γ_i denotes positive associative mating, and a negative one indicates that mating events occur between plants with opposite traits. Again, we estimated these γ_i coefficients jointly with the dispersal parameters (*a*, *b*) and the rate of external pollen flow (m).

Figure S1. Estimated relative fecundities of the outbred males vs. the inbred males, with 95% confidence interval. The relative fecundity of inbred males was set to one (hence no confidence interval is shown for this case).

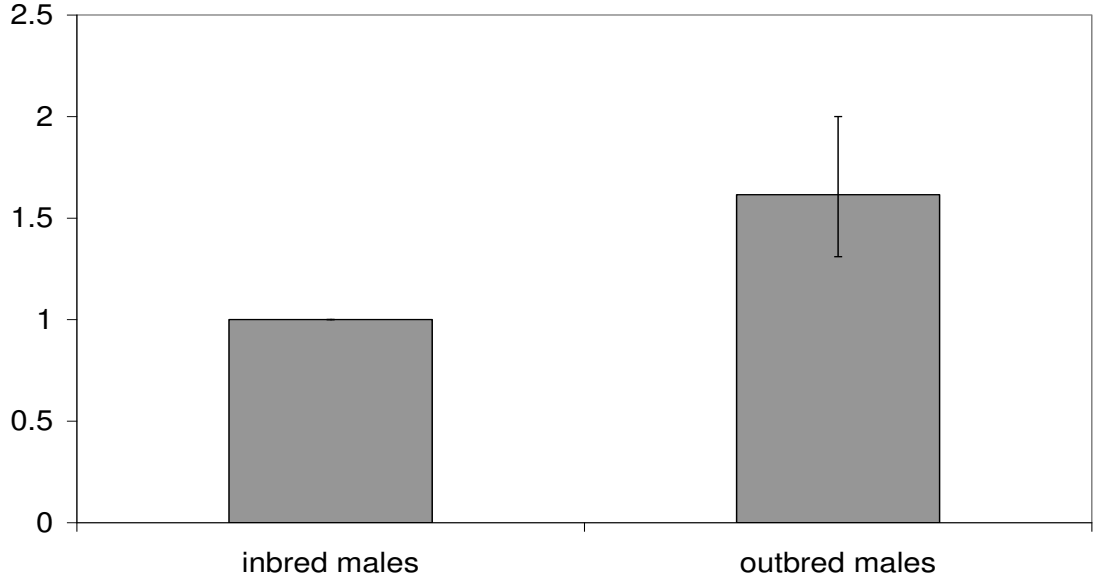


Figure S2. Estimated relative fecundities of the males as a function of their level of relatedness to the females. As paternity analysis methods can only estimate relative fecundities, the relative fecundity of the inbred males was set to one (hence no confidence interval is shown for this case).

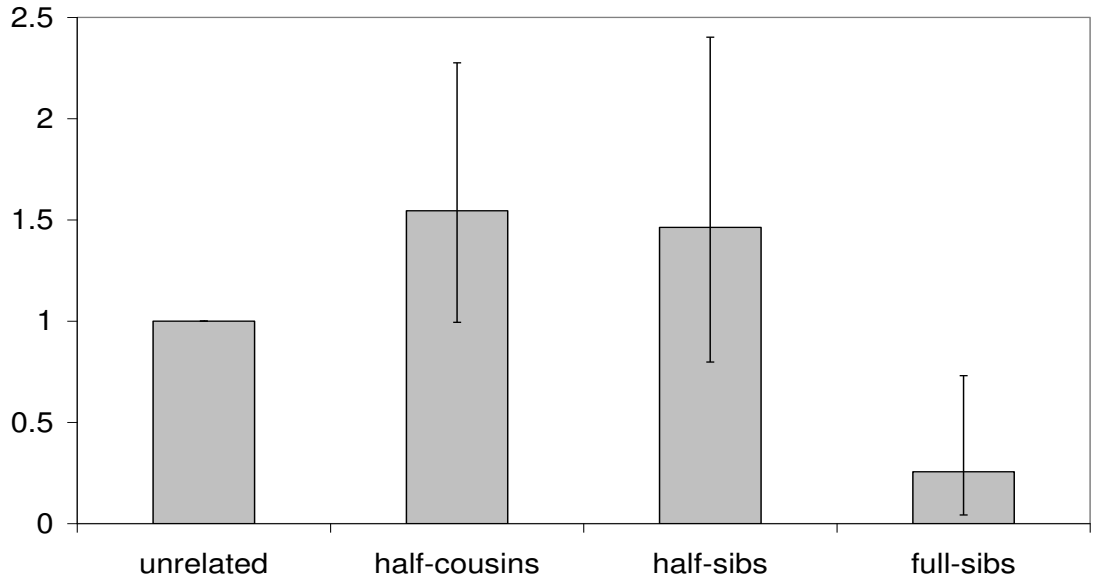


Figure S3. Estimated effects of the five considered traits on the relative male fecundity. The curves are drawn using equation (2) and the β coefficients given in Table 1.

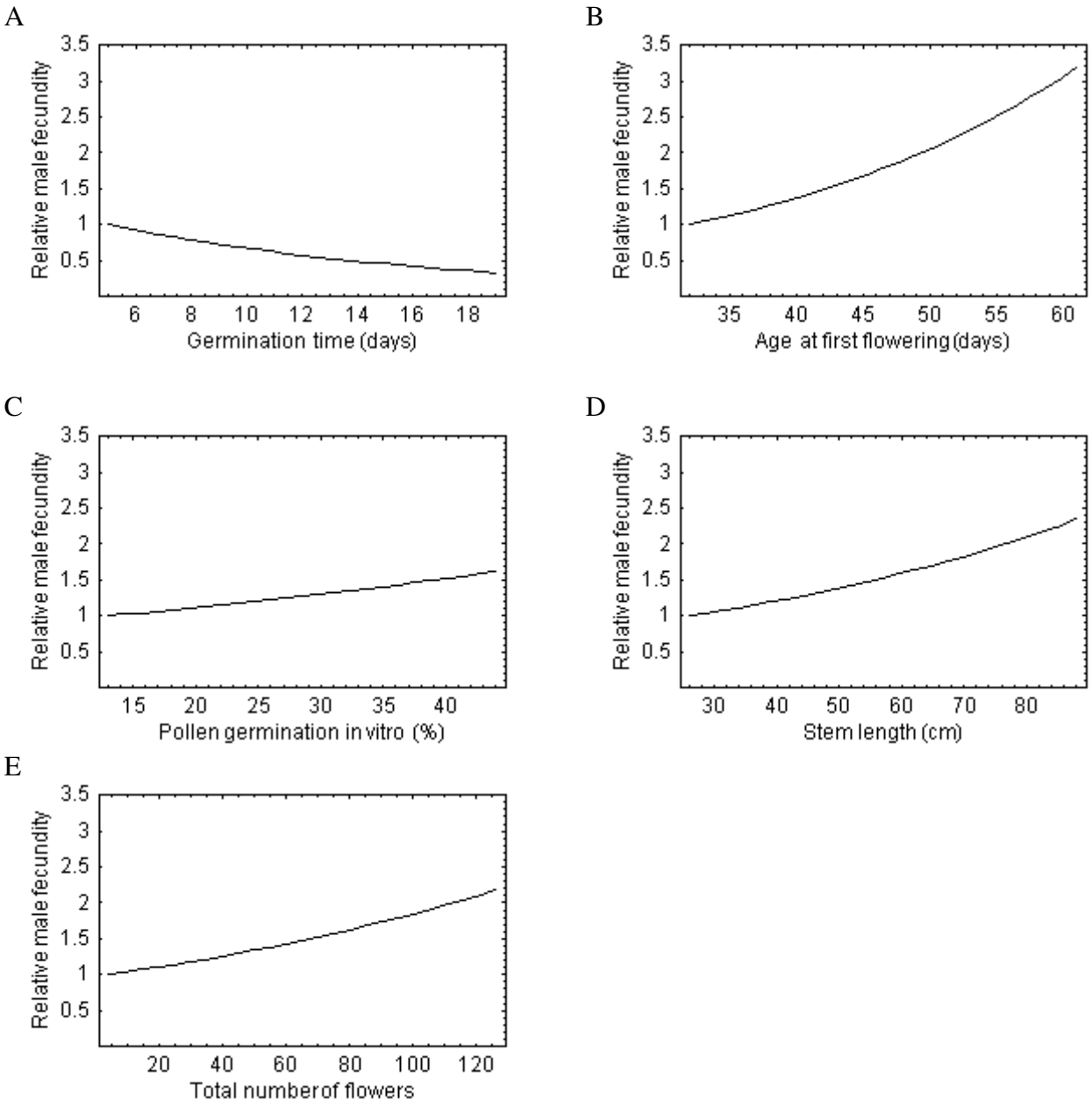


Figure S4

Graphical representation of the inferred dispersal kernel (Geometric distribution with parameters $a = 0.004$ and $b = 0.715$)